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Full Length Article

PTR-MS studies of the reactions of H_3O^+ with a number of deuterated volatile organic compounds and the subsequent sequential reactions of the primary product ions with water under normal and humid drift tube conditions: Implications for use of deuterated compounds for breath analysis



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ABSTRACT

Product ion distributions resulting from the primary reactions of H_3O^+ with nine D-labeled volatile organic compounds and the subsequent sequential reactions with H_2O have been determined using a Proton Transfer Reaction Time of Flight Mass Spectrometer (PTR-TOF 8000 (IONICON Analytik GmbH)) at various reduced electric field (E/N) values ranging from 80 up to 150 Td and for two different absolute humidity levels of air sample < 0.1% and 5%. The specific D-labeled compounds used in this study are acetone- d_6 , toluene- d_8 , benzene- d_6 , ethanol- d ($\text{C}_2\text{H}_5\text{OD}$), ethanol- d_2 ($\text{CH}_3\text{CD}_2\text{OH}$), ethanol- d_6 , 2-propanol- d_8 , 2-propanol- d_3 ($\text{CD}_3\text{CH}(\text{OH})\text{CH}_3$), and isoprene- d_5 ($\text{CH}_2\text{CHC}(\text{CD}_2)\text{CD}_3$). With the exception of the two 2-propanol compounds, non-dissociative proton transfer is the dominant primary reaction pathway. For 2-propanol- d_8 and 2-propanol- d_3 the major primary reaction channel involved is dissociative proton transfer. However, unlike their undeuterated counterparts, the primary product ions undergo subsequent deuterium/hydrogen isotope exchange reactions with the ever present water in the drift tube, the extent of which of course depends on the humidity within that tube. This exchange leads to the generation of various isotopologue product ions, the product ion branching percentages of which are also dependent on the humidity in the drift tube. This results in complex mass spectra and the distribution of product ions leads to issues of reduced sensitivity and accuracy. However, the effect of D/H exchange considerably varies between the compounds under study. In the case of acetone- d_6 it is very weak (< 1%), because the exchange process is not facile when the deuterium is in the methyl functional group. In comparison, the H_3O^+ /benzene- d_6 (C_6D_6) reaction and sequential reactions with water result in the production of the isotopologue ions $\text{C}_6\text{D}_n(\text{H}_{7-n})^+$ (where $n = 0-6$). Changing the value of E/N and/or the humidity in the drift tube considerably affects the amount of the isotope exchange reactions and hence the resulting sequential product ion distributions. An important conclusion of the findings from this work is that care must be taken in the choice of an exogenous deuterated compound for use in breath pharmacokinetic studies using proton transfer reaction mass spectrometry; otherwise the resulting D/H exchange processes impose interpretative problems.

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1. Introduction

The human body emits hundreds of volatile organic compounds (VOCs) via breath, skin emanation, and other bodily fluids such

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as urine, saliva, or sweat [1,2]. These compounds are frequently defined as the human volatilome [3]. While some of these VOCs are of endogenous origin, other can stem from exogenous sources such as diet, environmental exposure, or microbiota activity. Collectively, they form a chemical fingerprint containing information on biochemical processes ongoing in both healthy and diseased human organisms. It has been reported that pathological processes, such as metabolic disorders, cancer, or other diseases can influence the VOCs profile by producing new VOCs, or by altering the ratio of the VOCs that are produced normally by the human organism. This approach opens up new applied areas of research such as the potential use of trace volatiles for diagnosing, screening and monitoring diseases, tracking of microbiota activity, or exposure to environmental toxins [4–6]. Despite this huge potential, the use of these patterns within a clinical setting remains rather limited. The main unresolved issue is the poor understanding of the sources, behavior and metabolic fate of potential disease markers in the human organism.

Isotope labelling of volatiles could partially address these problems. ^{13}C -labeled compounds are commonly used in breath gas analysis. Important examples are the ^{13}C -urea breath test for *H. pylori* detection [7] and the ^{13}C -dextromethorphan/ ^{13}C -pantoprazol breath tests aiming at evaluating the enzyme activities [8,9]. In these tests breath $^{13}\text{CO}_2$ is targeted and the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio is used to evaluate the results. Alternatively, compounds of interest could be labeled with other isotopes such as ^{15}N , ^{18}O , or deuterium (D). In particular, the latter labelling received some attention in breath gas analysis to track metabolites of some VOCs with Proton Transfer Reaction - Mass Spectrometry (PTR-MS) [10,11], or to validate exhalation kinetics models [12]. However, these PTR-MS studies have only investigated deuterated ethanol or acetone. There have been no systematic studies of other deuterated compounds and in particular the effects of humidity in the drift (reaction) region of the PTR-MS instrument have not been investigated. Thus the reactions of hydronium ions with deuterated volatile organic compounds and subsequent reactions of the primary ions in the conditions of the PTR-TOF-MS reactors are poorly understood and the present study is intended to help fill this gap in our knowledge.

The main goal of this paper is to present details on the product ion distributions, which will be a combination of the primary product ion(s) resulting from the reactions of H_3O^+ and the subsequent sequential product ions involving nine deuterated organic compounds, over a reduced electric field range of 80–150 Td and at two different absolute humidity levels of < 0.1% and 5%, the latter approximately corresponding to that of exhaled breath using a PTR-TOF-MS instrument. The compounds selected for this investigation are acetone- d_6 , toluene- d_8 , benzene- d_6 , ethanol- d ($\text{C}_2\text{H}_5\text{OD}$), ethanol- d_2 ($\text{CH}_3\text{CD}_2\text{OH}$), ethanol- d_6 , 2-propanol- d_8 , 2-propanol- d_3 ($\text{CD}_3\text{CH}(\text{OH})\text{CH}_3$), and isoprene- d_5 ($\text{CH}_2\text{CHC}(\text{CD}_2)\text{CD}_3$).

Earlier examples of isotope exchange processes have been reported by Adams et al. [13] using a selected ion flow tube (SIFT). Of interest to this work, they reported the reactions of H_3O^+ with D_2O and D_3O^+ with H_2O . These two ion-molecule reactions were found to proceed with reaction rate coefficients close to their collisional values and that the product ion distributions are statistical owing to the production of thoroughly mixed intermediates (complete scrambling of the H and D atoms within the intermediate complex). Such scrambling is unlikely given the complexity of the analytes involved in this study, because in every intermediate deuterons/protons are attached to different atoms and not just to oxygen atoms, unlike the earlier work. However, it should be appreciated that unlike SIFT, PTR-MS is not designed to be a research instrument, but is an analytical device, and hence it is not always possible to clearly identify primary reaction processes owing to the more complex conditions found in the drift tube of a PTR-MS com-

pared to the cleaner chemical environment of the flow tube in a SIFT.

With regards to SIFT studies, and in relation to the biological aspects presented in this study, we comment that there has been a SIFT H_3O^+ investigation of the kinetics and isotope patterns of ethanol and acetaldehyde head space emissions from yeast fermentation of glucose-6,6- d_2 by Smith et al. [14].

2. Experimental

2.1. Materials and standard mixtures

Single-compound calibration mixtures were prepared from pure liquid substances. The majority of the pure substances were purchased from Sigma-Aldrich (Austria); acetone- d_6 (99.9%), toluene- d_8 (99%), benzene- d_6 (99.6%), ethanol- d (99%), ethanol- d_2 (98%), ethanol- d_6 (99.5%), 2-propanol- d_8 (99.5%), and 2-propanol- d_3 (98%). Isoprene- d_5 (98%) was obtained from Campro Scientific GmbH (Germany).

Gas standard mixtures were prepared using the procedure outlined in Mochalski et al. [15]. The product ion distributions were investigated using 2 distinct concentrations of each compound to ensure that there were no secondary ion-molecule reactions with the analyte, i.e. that the distributions of the product ion channels did not change in high purity air at concentrations of approximately 50 and 100 ppb and at two absolute humidity levels of < 0.1% and 5%, the latter approximately corresponding to that of exhaled breath.

2.2. PTR-TOF-MS analysis

PTR-MS is an analytical device that has applications ranging from atmospheric chemistry through to homeland security [16–22], but it really comes into its own for monitoring rapid changes in volatile concentrations via its real-time capabilities. These real-time analytical techniques are particularly attractive for tracking rapid short-time changes in the human volatilome [14,15], which can provide invaluable information on normal and abnormal processes occurring in the body [12,23–27]. This is due to its versatility, excellent sensitivity and (for ToF MS) high mass resolving power (up to 5000 $m/\Delta m$). In particular, the last feature supports studies to separate isobaric compounds producing nominally the same m/z product ions.

The H_3O^+ reactions with the deuterated compounds and the subsequent reactions of the primary product ions with H_2O were investigated using a PTR-TOF 8000 (Ionicon Analytik GmbH). The settings of the ion source used in this study were as follows: ion source current 3 mA, source voltage 140 V, source-out voltage 30 V, and source valve opening 50%. At these values and a purified air sample in the drift tube operating at 130 Td, a temperature of 60 °C and a pressure of 2.4 mbar, the major other reagent ions in the drift tube relative to that of the H_3O^+ reagent ion were NO^+ with 0.2–0.3%, O_2^+ with 2% and $\text{H}_3\text{O}^+ \cdot \text{H}_2\text{O}$ with 3%. However, at low reduced electric fields (< 100 Td), $\text{H}_3\text{O}^+ \cdot \text{H}_2\text{O}$, formed from an association reaction of H_3O^+ with H_2O , becomes the dominant reagent ion.

Note that a dry buffer gas in the drift tube of a PTR-MS instrument does not mean that it is operating under dry conditions, because of diffusion of water vapour from the hollow cathode. We will, therefore, refer to this as “normal” operating conditions. When a water saturated buffer gas is used, we will refer to that as operating the drift tube under “humid” conditions.

The product ion distributions were investigated for eight distinct E/N ratios in the reaction tube ranging from 80 to 150 Td with incremental changes of 10 Td by adjusting the drift tube voltage. The ion mass (m/z) calibration was regularly checked using the

presence of the impurity ions of known m/z values, namely: $\text{H}_3^{18}\text{O}^+$ (21.0221) and NO_2^+ (45.9924).

The standard mixtures of deuterated compounds were introduced into the drift tube of the PTR-TOF-MS instrument at a steady flow rate of 20 mL/min via a two-meter-long, heated (60 °C) PEEK (Polyetheretherketone) transfer line reducing the surface adsorption of water vapor and the deuterated compounds under study.

The mass spectral scans converted from the drift times of the ions in the TOF analyzer ranged from approximately m/z 2.7 to 500 and were acquired in a time of 30 s by co-adding 750 000 single 40- μs long TOF-MS extractions recorded at a sampling frequency of 10 GHz. The actual mass resolution in the present experiment obtained from the detected peaks was ≈ 4300 at m/z 100. The total duration of a single measurement was 3 min, which corresponds to 6 mass spectra acquired per single E/N ratio. The average of the ion signal levels at each m/z value from these 6 spectra was used to calculate the percentages of the product ions for each of the H_3O^+ /deuterated VOC reactions.

3. Results

Table 1 lists the percentage distributions of the product ions resulting from the primary reactions of H_3O^+ with the 9 deuterated volatiles included in this study and the subsequent sequential product ion-water reactions. Thus, the product ion distributions include the combination of primary ions and the product ions resulting from a series of sequential reactions with water in the drift tube of the PTR-MS under the operational conditions used in this study. These percentages refer to the average of intensities obtained for the two distinct concentrations using the average of 6 spectra for each deuterated compound.

A review of Table 1 immediately reveals that despite the single ionization mechanism via exothermic proton transfer the product ion distributions are relatively complex. This is due to the sequential deuterium-hydrogen exchange reactions between product ions and neutral water in the drift tube leading to several isotopologue ions. However, the amount of exchange considerably varies between compounds under study and depends on reaction time (which is inversely proportional to the reduced electric field) and humidity. This is clearly demonstrated for toluene, for both the dry and humid case the protonated parent $\text{C}_7\text{D}_8\text{H}^+$ percentage increases with increasing E/N owing to the reduced reaction time for secondary reactions, but in the humid sample the percentages are lower owing to the increased concentration of water in the drift tube available for D/H exchange. Reaction of $\text{C}_7\text{D}_n\text{H}_{9-n}^+$ ($n=4-8$) with water terminates once the deuterium atoms in the benzene ring have been exchanged ($n=3$), i.e. exchange of the deuterium atoms on the methyl group does not take place.

Our results show that the primary reaction does not result in a statistical decomposition. For example, in the case of toluene- d_8 , if complete scrambling of the H and D atoms within the intermediate complex $\text{C}_7\text{D}_8\text{H}_3\text{O}^+$ occurred, the primary reaction of H_3O^+ with toluene- d_8 would proceed via three channels leading to $\text{C}_7\text{D}_8\text{H}^+ + \text{H}_2\text{O}$, $\text{C}_7\text{D}_7\text{H}_2^+ + \text{HDO}$, and $\text{C}_7\text{D}_6\text{H}_3^+ + \text{D}_2\text{O}$, with probabilities of 5.5%, 43.6% and 50.9%, respectively. That the first channel is found to dominate for the dry air measurements, with a probability of 48%, implies that following proton transfer from H_3O^+ , the protonated toluene- d_8 rapidly dissociates, i.e. the dissociation lifetime leading to $\text{C}_7\text{D}_8\text{H}^+ + \text{H}_2\text{O}$ is far less than the lifetime of the intermediate complex. Although some scrambling cannot be ruled out, the results suggest that proton transfer is the dominant primary reaction process, and that all other observed product ions are predominantly a result of sequential reactions with water in the drift tube. The same is true for benzene- d_6 , for which the product ion distributions for the primary reaction occurred via statistical decomposition. The H_3O^+ /benzene- d_6 (C_6D_6) reaction and subse-

quent reactions with H_2O in the drift tube results in the observed isotopologue $\text{C}_6\text{D}_n\text{H}_{7-n}^+$ ($n=0-6$).

In the case of fully deuterated acetone, isotopic exchange is not facile with the methyl groups and hence the only dominant ion observed is the protonated parent in both dry and humid conditions. The product ion distributions of the H_3O^+ /isoprene- d_5 reaction is more complicated owing to some fragmentation following proton transfer. However the data show that isotope exchange is not significant, as is apparent from the comparison of the dry and humid air samples, with protonated isoprene- d_5 being the dominating ion with abundances ranging from 80% to 55% for 80 Td and 150 Td respectively. The main isotope exchange channel corresponds to the single D/H exchange and its abundance is spread around 10% for all investigated reduced electric field values, and the multiple D/H exchange channels are weak ($< 3\%$).

In a PTR-Quad-MS study by Brown et al. [21], it is reported that for 2-propanol C_3H_7^+ is a major product ion. Similarly, for 2-propanol- d_8 , the dominant product ion observed here is C_3D_7^+ , resulting from the loss of HDO from the protonated parent. However, owing to D/H exchange with the water in the drift tube of the PTR-MS, $\text{C}_3\text{D}_n\text{H}_{7-n}^+$ ($n=0-3, 5-7$) product ions are observed, thereby reducing the intensity of the primary product ion. $\text{C}_3\text{D}_4\text{H}_3^+$ must be formed, but it is not reported in the table because no significant intensity ($< 1\%$) of this ion was observed. Similarly, for 2-propanol- d_3 , $\text{C}_3\text{D}_3\text{H}_4^+$ is the dominant ion and then sequential isotope exchange results ultimately in C_3H_7^+ . The protonated parent is observed, but only with a small intensity in the dry air case, and the D/H exchange results in observable intensities of $\text{C}_3\text{D}_3\text{H}_6\text{O}^+$ and $\text{C}_3\text{DH}_8\text{O}^+$ for 2-propanol- d_3 and $\text{C}_3\text{D}_7\text{H}_2\text{O}^+$ for 2-propanol- d_8 . For the higher humidity studies, and in particular at low E/N values, a dramatic rise in the associated branching percentages is observed for these ions. This is because of the higher concentration of the $\text{H}_3\text{O}^+ \cdot \text{H}_2\text{O}$ reagent ions in the drift tube resulting from the humid air sample at any given E/N compared to the dry air sample. Proton transfer from $\text{H}_3\text{O}^+ \cdot \text{H}_2\text{O}$ to the 2-propanol- d_3 and d_8 will be less energetic than from H_3O^+ , resulting in less fragmentation. Given that the proton affinity of 2-propanol (793 kJ mol^{-1}) is slightly less than that of the water dimer $(\text{H}_2\text{O})_2$ (808 kJ mol^{-1}) [22], proton transfer is driven by the reduced electric field. In the work by Brown et al. with 2-propanol, at high E/N ($> 115 \text{ Td}$) the product ions C_3H_5^+ and C_3H_3^+ were observed with reasonable intensities. In this study, only 2-propanol- d_3 resulted in fragment ions beyond $\text{C}_3\text{D}_n\text{H}_{7-n}^+$ ($n=0-3$) with reasonable intensities. This illustrates how important it is to determine product ion branching percentages for a given PTR-MS instrument.

For the deuterated ethanol, the product ions observed are predominantly $\text{C}_2\text{D}_m\text{H}_n\text{O}^+$, where $m+n=7$. For ethanol- d , only $m=0$ and $n=7$ is observed, i.e. no $\text{C}_2\text{H}_5\text{ODH}^+$ is observed with any significant intensity. Obviously it is formed, but D/H exchange is facile when the deuterium atom is bound to oxygen in the hydroxyl group. This is also the reason why no $\text{C}_2\text{D}_5\text{ODH}^+$ is observed from the reaction of H_3O^+ with ethanol- d_6 . For ethanol- d_2 , a dissociative proton transfer product ion channel is observed resulting in $\text{C}_2\text{D}_n\text{H}_{5-n}^+$ ($n=1-2$). This agrees well with the work of Brown et al. [21]. However, Brown et al. in addition reported significant product ion branching percentages for $[\text{ROH}-\text{H}_2]\text{H}^+$ (e.g. at an E/N of 138 Td they give a percentage branching percentage of 17% for this product ion). We observe the equivalent product ion for ethanol- d but only significantly at the maximum E/N used in this study, giving a product ion branching percentage of only 6% for both the dry and humid air samples. Similarly, for ethanol- d_6 , the product ion $\text{C}_2\text{D}_4\text{HO}^+$ resulting from the elimination of HD following proton transfer is observed, with a product ion branching percentage that reaches 23% at an E/N of 150 Td in the dry air sample. For the humid air sample its intensity is reduced to 14% at the highest E/N value. This could be the result of changes in

Table 1
Product ion distributions resulting from the reactions of H_3O^+ with 8 deuterated compounds and subsequent reactions with water using dry and humid (AH 5%) air samples as the buffer gas in the drift (reaction) tube of an IONICON Analytik GmbH PTR-TOF 8000 instrument for E/N values ranging from 80 to 150 Td.

Compound Formula purity CAS	Reaction channel	Dry air 0%AH [Td]								Humid air 5% AH [Td]								Expected m/z [Th]	Measured m/z [Th]	Error [mTh]
		80	90	100	110	120	130	140	150	80	90	100	110	120	130	140	150			
Toluene- d_8 C_7D_8 99% 2037-26-5	$\text{C}_7\text{D}_8\text{H}^+$	48	58	66	72	75	77	79	79	4	4	5	9	16	24	31	38	101.1206	101.1195	1.1
	$\text{C}_7\text{D}_7\text{H}_2^+$	33	30	26	23	20	19	18	17	6	7	10	17	25	31	34	35	100.1144	100.1133	1.1
	$\text{C}_7\text{D}_6\text{H}_3^+$	14	10	6	4	3	2	2	2	3	8	16	24	27	26	22	19	99.1081	99.1072	0.9
	$\text{C}_7\text{D}_5\text{H}_4^+$	4	2	1	1	1	1	0	0	7	18	26	27	21	14	10	7	98.1018	98.1008	1.0
	$\text{C}_7\text{D}_4\text{H}_5^+$	1	1	1	1	1	1	1	1	24	32	28	18	10	5	3	2	97.0955	97.0936	1.9
Benzene- d_6 C_6D_6 99.6% 1076-43-3	$\text{C}_7\text{D}_3\text{H}_6^+$	0	0	0	0	0	0	0	0	56	32	15	6	2	1	1	0	96.0893	96.0883	1.0
	$\text{C}_6\text{D}_6\text{H}^+$	7	10	13	18	22	27	31	35	2	1	1	1	2	3	3	4	85.0924	85.0916	0.8
	$\text{C}_6\text{D}_5\text{H}_2^+$	8	11	15	20	25	29	31	33	7	6	4	3	3	4	5	7	84.0862	84.0851	1.1
	$\text{C}_6\text{D}_4\text{H}_3^+$	9	13	18	22	23	23	22	20	1	1	1	1	2	4	6	8	83.0799	83.079	0.9
	$\text{C}_6\text{D}_3\text{H}_4^+$	13	18	20	19	17	14	11	9	0	0	0	1	3	5	8	12	82.0736	82.0728	0.8
Acetone- d_6 $\text{C}_3\text{D}_6\text{O}$ 99.9% 666-52-4	$\text{C}_6\text{D}_2\text{H}_5^+$	20	21	18	14	9	6	4	3	2	2	2	3	5	9	15	20	81.0673	81.0665	0.8
	C_6DH_6^+	25	18	11	6	3	2	1	1	1	1	1	5	12	20	26	26	80.0611	80.0605	0.6
	C_6H_7^+	18	8	3	1	0	0	0	0	87	89	90	86	73	56	37	22	79.0548	79.0540	0.8
	$\text{C}_3\text{D}_6\text{HO}^+$	98	98	98	98	98	98	98	98	98	98	98	98	98	98	98	98	65.0874	65.0870	0.4
	$\text{C}_3\text{D}_5\text{H}_2\text{O}^+$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	64.0810	64.0808	0.2
Isoprene- d_5 $\text{CH}_2\text{CHC}(\text{CD}_2)\text{CD}_3$ 98%	$\text{C}_2\text{D}_3\text{O}^+$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	46.0372	46.0366	0.6
	$\text{C}_5\text{D}_5\text{H}_4^+$	79	79	79	79	78	75	67	55	74	78	79	80	80	79	76	69	74.1018	74.1014	0.4
	$\text{C}_5\text{D}_4\text{H}_5^+$	11	11	11	11	11	10	9	8	15	13	12	12	11	11	10	9	73.0955	73.0941	1.4
	$\text{C}_5\text{D}_3\text{H}_6^+$	3	3	3	3	3	3	3	3	6	4	3	3	3	3	3	3	72.0893	72.0882	1.1
	$\text{C}_5\text{D}_2\text{H}_7^+$	1	1	1	1	1	1	2	2	3	2	1	1	1	1	1	2	71.0830	71.0828	0.2
2-propanol- d_8 $\text{C}_3\text{D}_8\text{O}$ 99.5% 22739-76-0	$\text{C}_3\text{D}_4\text{H}^+$	1	1	1	1	1	2	3	4	1	1	1	1	1	1	1	1	45.0642	45.0649	0.7
	$\text{C}_3\text{D}_3\text{H}_2^+$	3	3	3	4	4	5	8	13	2	2	2	2	2	3	5	5	44.058	44.0589	0.9
	$\text{C}_3\text{D}_2\text{H}_3^+$	0	0	0	0	0	2	5	11	0	0	0	0	0	1	2	5	43.0517	43.0528	1.1
	C_3DH_4^+	1	1	1	1	1	1	3	5	0	0	0	0	1	1	2	6	42.0454	42.0454	0.1
	$\text{C}_3\text{D}_7\text{H}_2\text{O}^+$	2	1	0	0	0	0	0	0	20	15	8	4	2	1	1	1	68.1093	68.1091	0.2
2-propanol- d_3 $\text{CD}_3\text{CH}(\text{OH})\text{CH}_3$ 98% 84809-71-2	C_3D_7^+	65	63	62	62	60	55	47	40	63	55	36	25	22	23	25	27	50.0987	50.0982	0.5
	$\text{C}_3\text{D}_6\text{H}^+$	21	23	23	23	22	19	14	9	11	13	16	19	23	25	25	23	49.0924	49.0923	0.1
	$\text{C}_3\text{D}_5\text{H}_2^+$	5	5	5	5	5	4	2	1	2	5	14	21	24	24	21	15	48.0862	48.0858	0.4
	$\text{C}_3\text{D}_3\text{H}_4^+$	4	5	5	6	8	13	21	28	2	5	13	16	15	13	11	8	46.0736	46.0718	1.8
	$\text{C}_3\text{D}_2\text{H}_5^+$	1	1	1	2	3	5	10	14	1	4	8	9	8	7	7	8	45.0673	45.0652	2.1
Ethanol- d_6 $\text{C}_2\text{D}_5\text{OD}$ 99.6% 1516-08-1	C_3DH_6^+	0	0	0	0	1	2	3	5	1	2	4	4	4	6	9	9	44.0611	44.0590	2.1
	C_3H_7^+	2	2	2	2	2	2	2	2	1	1	2	3	3	3	6	10	43.0548	43.0554	0.6
	$\text{C}_3\text{D}_3\text{H}_6\text{O}^+$	2	1	0	0	0	0	0	0	25	18	9	4	2	1	1	0	64.0842	64.0838	0.4
	$\text{C}_3\text{DH}_8\text{O}^+$	5	5	5	5	5	5	5	6	6	9	9	8	6	6	6	7	62.0716	62.0684	3.2
	$\text{C}_3\text{D}_3\text{H}_4^+$	73	73	72	71	68	58	45	34	57	50	36	32	33	35	36	34	46.0736	46.0732	0.4
Ethanol- d_2 $\text{C}_2\text{D}_2\text{H}_4\text{O}(\text{H}_3\text{O})^+$ 98% 1859-09-2	$\text{C}_3\text{D}_2\text{H}_5^+$	11	11	11	11	10	8	6	3	6	10	20	27	30	29	23	16	45.0673	45.0667	0.6
	C_3DH_6^+	3	3	4	4	5	8	10	12	2	7	15	18	17	15	11	7	44.061	44.0585	2.5
	C_3H_7^+	5	6	6	6	8	13	20	22	2	3	5	6	6	6	8	10	43.0547	43.0532	1.5
	C_3H_6^+	1	1	1	1	2	5	8	9	1	1	2	2	3	4	8	13	42.047	42.0465	0.5
	C_3H_5^+	0	0	0	0	1	1	3	7	0	1	1	1	2	3	6	10	41.0391	41.0393	0.2
Ethanol- d $\text{C}_2\text{H}_5\text{OD}$ 99% 925-93-9	C_3H_4^+	0	0	0	0	0	1	3	7	1	2	2	2	1	1	1	3	40.0313	40.0300	1.3
	$\text{C}_2\text{D}_5\text{HO}(\text{H}_3\text{O})^+$	0	0	0	0	0	0	0	0	2	2	1	1	0	0	0	0	70.0916	70.0922	0.6
	$\text{C}_2\text{D}_5\text{OD}$	75	67	62	66	70	69	66	65	83	78	62	49	54	63	68	70	52.0811	52.0808	0.3
	$\text{C}_2\text{D}_4\text{H}_3\text{O}^+$	8	13	17	14	8	5	4	3	4	7	14	18	18	15	11	9	51.0748	51.0743	0.5
	$\text{C}_2\text{D}_3\text{H}_4\text{O}^+$	3	5	5	3	1	1	0	0	1	3	10	15	11	6	3	2	50.0685	50.0680	0.5
Ethanol- d $\text{C}_2\text{H}_5\text{O}^+$ 99% 925-93-9	$\text{C}_2\text{D}_4\text{H}_2\text{O}^+$	2	3	4	7	12	17	21	23	1	1	3	8	8	8	11	14	49.0591	49.0585	0.6
	C_2D_3^+	4	3	3	3	4	4	5	5	4	4	3	3	3	4	4	5	34.0705	34.0697	0.8
	$\text{C}_2\text{D}_4\text{H}^+$	3	3	3	1	0	0	0	0	2	2	2	1	0	0	0	0	33.0858	33.0621	24*
	$\text{C}_2\text{D}_3\text{H}_2^+$	3	4	4	5	4	4	4	4	2	2	3	3	3	3	3	3	32.0580	32.0567	1.3
	$\text{C}_2\text{D}_2\text{H}_3^+$	1	1	2	1	1	1	1	0	0	0	1	2	2	1	1	0	31.0517	31.0509	0.8
Ethanol- d $\text{C}_2\text{H}_5\text{O}^+$ 99% 925-93-9	$\text{C}_2\text{D}_2\text{H}_4\text{O}(\text{H}_3\text{O})^+$	0	0	0	0	0	0	0	0	7	6	5	4	3	3	3	3	67.0728	67.0715	1.3
	$\text{C}_2\text{D}_2\text{H}_5\text{O}^+$	78	76	75	76	76	75	75	75	83	83	81	74	67	70	74	77	49.0622	49.0611	1.1
	$\text{C}_2\text{DH}_6\text{O}^+$	5	6	6	5	3	2	2	2	2	3	5	13	20	18	14	12	48.0560	48.0548	1.2
	$\text{C}_2\text{D}_2\text{H}_3^+$	10	10	10	11	13	15	15	17	6	6	6	6	6	6	6	7	31.0517	31.0502	1.5
	C_2DH_4^+	6	8	9	9	9	8	8	8	2	2	2	3	4	3	3	2	30.0454	30.0439	1.5
Ethanol- d $\text{C}_2\text{H}_5\text{O}^+$ 99% 925-93-9	$\text{C}_2\text{H}_6\text{O}(\text{H}_3\text{O})^+$	1	0	0	0	0	0	0	0	6	5	4	3	3	2	1	1	65.0595	65.0603	0.8
	$\text{C}_2\text{H}_7\text{O}^+$	83	82	82	81	81	80	81	80	86	86	86	86	85	84	81	79	47.0497	47.0490	0.7
	$\text{C}_2\text{H}_5\text{O}^+$	0	0	0	1	2	3	4	6	0	0	0	0	1	2	4	6	45.0340	45.0336	0.4
	C_2H_5^+	16	17	18	18	18	17	15	13	8	9	9	10	11	12	13	14	29.0391	29.0388	0.3

* peak overlapped by a background methanol peak.

the effective temperature of the reagent ion-ethanol collision for a given E/N caused by the higher humidity present in the drift tube.

4. Discussion and conclusions

The rationale behind this study was to identify the product ions resulting from the reactions of H_3O^+ ions with nine D-labeled volatile organic compounds and sequential reactions with water in the drift tube of a PTR-TOF-MS instrument, and thus to evaluate their usefulness for studies on the human volatilome. This was done for eight different E/N values in the drift tube reactor ranging from 80 to 150 Td and for two different absolute humidity levels of the buffer air < 0.1% and 5%. With the exception of those compounds for which the deuterium atoms form a methyl group, following the primary proton transfer, sequential D/H substitution with water in the drift tube leads to complicated mass spectra, which complicates analysis for breath research applications using PTR-MS. Moreover, changes of E/N in the drift tube modify the product ion distribution. In most of the reactions, higher E/N facilitates multiple D/H isotope exchange and promotes the formation of lighter isotopologues. Humidity has a strong effect on the product ion distributions and promotes the deuterium transfer from the molecule otherwise observed in the dry air sample measurements.

When the deuterium atoms are bonded to the carbon atom of an alcohol molecule the isotope exchange reaction is less probable. This is well illustrated by the product ion distributions of ethanol- d_2 and 2-propanol- d_3 , where a relatively small fraction of the parent ion undergoes this reaction and protonated ethanol- d_2 ($\text{C}_2\text{D}_2\text{H}_5\text{O}^+$) and $\text{C}_3\text{D}_3\text{H}_4^+$ (from the $\text{C}_3\text{D}_3\text{H}_6\text{O}^+$ parent) are the dominant reaction channels, respectively. The D/H isotope exchange reaction becomes more efficient with increasing E/N . For example, in the ethanol- d_6 product ion distribution the abundance of the $\text{C}_2\text{D}_2\text{H}_5\text{O}^+$ ion is only 3% at an E/N of 80 Td, whilst at 150 Td it is at a level of 25%. Consequently, by varying E/N the isotope exchange reactions can be suppressed accordingly, thus simplifying the resulting spectrum.

A key point from this investigation is that the sequential D/H isotope exchange processes of the primary product ions, resulting from the initial reactions of H_3O^+ with deuterated compounds, with neutral water in the drift (reaction) tube of a PTR-MS instrument will in most cases result in complex mass spectra, thereby reducing the intensity of the primary product ions, and hence leading to a reduction in detection sensitivity and possible accuracy. This limits somewhat the application of D-labeled species when using PTR-MS, because the drift tube will always have water present owing to diffusion from the ion source, independent as to how dry is the sample air entering the drift tube. This is particularly troubling for breath research. Certainly, the increased humidity in breath samples compared to air samples will have a considerable effect on the product ion distributions and impose interpretative problems, but there is an additional problem of the variability of humidity that occurs in breath samples. Therefore, the monitoring of deuterated compounds and their metabolites in breath will be problematic and challenging, especially when the metabolites are unknown. Thus, in conclusion, particular care has to be taken during the study design when considering employing D-labeled substrates for breath research using a PTR-MS instrument as the analytical tool owing to the substantial H/D exchange that may occur in the drift tube.

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